

H. Xu · R. M. Robertson

Effects of temperature on properties of flight neurons in the locust

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Abstract High ambient temperatures increase the wing-beat frequency in flying locusts, *Locusta migratoria*. We investigated parameters of circuit and cellular properties of flight motoneurons at temperatures permissive for flight (20–40 °C). As the thoracic temperature increased motoneuronal conduction velocity increased from an average of 4.40 m/s at 25 °C to 6.73 m/s at 35 °C, and the membrane time constant decreased from 11.45 ms to 7.52 ms. These property changes may increase locust wing-beat frequency by affecting the temporal summation of inputs to flight neurons in the central circuitry. Increases in thoracic temperature from 25–35 °C also resulted in a hyperpolarization of the resting membrane potentials of flight motoneurons from an average of –41.1 mV to –47.5 mV, and a decrease of input resistances from an average of 3.45 M Ω to 2.00 M Ω . Temperature affected the measured input resistance both by affecting membrane properties, and by altering synaptic input. We suggest that the increase in conduction velocity ($Q_{10}=1.53$) and the decrease of membrane time constant ($Q_{10}=0.62$) would more than account for the wing-beat frequency increase ($Q_{10}=1.15$). Hyperpolarization of the resting membrane potential ($Q_{10}=1.18$) and reduction in input resistance ($Q_{10}=0.54$) may be involved in automatic compensation of temperature effects.

Key words Locust · Temperature · Flight · CPG
Neuronal properties · Intracellular recording

Abbreviations ANOVA analysis of variance
CPG central pattern generator
DL dorsal longitudinal muscles
EMG electromyographic · MN motoneuron
PSP post synaptic potential · Q_{10} temperature coefficient
RMP resting membrane potential
S.D. standard deviation · SR stretch receptor

Introduction

Successful motor programs are those which can adapt to a fluctuating set of internal and external conditions to produce appropriate output. It is increasingly apparent that such flexibility can reside in the properties of single, anatomically-defined, neuronal circuits (Harris-Warrick and Marder 1991), and several examples of this form of short-term plasticity now exist (e.g. Hooper and Moulins 1989; Wolf and Pearson 1989; Möhl 1989, 1993). An exciting challenge is to define the neural mechanisms controlling reconfiguration of functional circuits.

One form of flexibility lies in the ability to adjust the properties of the circuit to alter the output and thus cope with different conditions. Flexibility is also required to maintain a stable output by compensating for changes imposed by variations in the environment. An environmental variable which can have considerable impact on the operation of neuronal circuits, and the form and existence of subsequent behaviour, is temperature. All neural phenomena are affected by temperature to a greater or lesser extent (Montgomery and Macdonald 1990; Janssen 1992). In most cases this is due to simple effects of temperature on basic membrane properties, such as the dynamic properties of ion channels (Dilger et al. 1991). However, many opposing neuronal processes are accelerated by increases in temperature and the consequences for motor patterns or behaviour become difficult to predict.

Locomotor performance in many poikilotherms is critically dependent upon ambient temperature (Band 1990; Full and Tullis 1990). Indeed, rhythmic behaviours and motor patterns are typically highly temperature dependent (e.g. heartbeat frequency in leeches – Arbas and Calabrese 1984; parapodial swimming in *Aplysia* – Von Der Porten et al. 1982; teleost and ascidian swimming – Batty et al. 1991; pyloric contractions in lobsters – Johnson et al. 1991, 1992). In contrast, locust flight performance is comparatively resistant to temperature fluctuation throughout a relatively wide range. Flight is an energetically demanding activity and it is accomplished most

H. Xu (✉) · R. M. Robertson
Department of Biology, Queen's University, Kingston, Ontario,
K7L 3N6, Canada

efficiently at high operating temperatures. To achieve this some insects have the ability to regulate thoracic temperature and maintain it in excess of ambient by as much as 35 °C (e.g. Morgan 1987). Locusts can generate a thoracic temperature of 5–10 °C above ambient (Weis-Fogh 1964) but they do not show a tight regulation of thoracic temperature which is thus likely to vary throughout the 24–42 °C range permissible for flight (Weis-Fogh 1956; Neville and Weis-Fogh 1963). One measure of flight performance, wing-beat frequency, changes minimally with temperature in locusts (Weis-Fogh 1956; Foster and Robertson 1992) in spite of much greater effects of temperature on synaptic parameters in the flight circuit (Robertson 1993). This temperature dependence is minimal, even in comparison with relatively mild temperature effects on the wing-beat frequency of other insect species (May 1981; Josephson 1981). This implies that compensatory mechanisms exist within the flight system of locusts to stabilize the output frequency.

The flight motor pattern of intact locusts is generated by neuronal circuitry that includes central and peripheral components (Robertson 1986; Pearson and Ramirez 1990; Wolf 1993). Although it is clear that phasic proprioceptive feedback sets the final wingbeat frequency in intact animals (Pearson and Ramirez 1990), preliminary evidence suggests that the effect of temperature on wing-beat frequency is likely mediated by its effect on the central component (Foster and Robertson 1992). Deafferentation decreases the frequency of the rhythm (Wilson 1961) but many of the characteristics of the intact motor pattern remain evident (Stevenson and Kutsch 1987). Analyses of numerous central pattern generators (CPGs) has shown that any particular system tends to be constructed as an idiosyncratic selection of common building blocks of cellular, synaptic and network properties (Getting 1989), which are common to both vertebrates and invertebrates (Pearson 1993). The central circuit underlying locust flight has not been completely described. Flight interneurons have been identified (reviewed in Robertson 1986, 1989) and some have been shown to support plateau potentials which may contribute to rhythm generation in the system (Ramirez and Pearson 1991a, b). Synaptic interactions include classic short latency excitatory and inhibitory interactions and a delayed excitatory interaction that is probably mediated disynaptically (Robertson and Pearson 1985) via non-spiking interactions (Robertson 1991). The complete flight system is under neuromodulatory control at different levels (Orchard et al. 1993). A description of the effects of temperature on the neural building blocks of the flight system is incomplete. In the locust, temperature affects firing thresholds, the relationship between stimulus current and firing frequency, and other intrinsic membrane properties (Heitler et al. 1977; Abrams and Pearson 1982; Burrows 1989; Simmons 1990). Increases in temperature also increase or decrease the amplitude of postsynaptic potentials (PSPs) depending upon the range of temperatures used (Abrams and Pearson 1982; Burrows 1989). Above about 24 °C, i.e. within the range

permissive for flight, a temperature increase causes a decrease in PSP amplitude and decreases in temporal parameters of the PSP (Robertson 1993). A portion of the decrease in synaptic latency with increased temperature is attributable to a predicted increase in conduction velocity, as has been noted elsewhere (Reichert and Rowell 1985; Burrows 1989). The discharge frequency of mechanosensory neurons is markedly affected by temperature (Miles 1985; Pfau et al. 1989) although, for wind-sensitive hairs on the head, some of the effects of temperature are compensated for at the level of first order wind-sensitive interneurons (Miles 1992).

We describe quantitatively the effects of temperature on cellular properties of flight neurons and on parameters of bursting activity during expression of the flight rhythm. This is a preliminary step in an investigation of the neural mechanisms involved in temperature compensation in the flight system. One mechanism underlying compensation may be that the basic thermal properties of flight neurons are different from other neurons in the locust thoracic nervous system. This possibility is refuted here.

Materials and methods

Adult male *Locusta migratoria* at least 10 days past the final molt were collected from a crowded breeding colony in the Department of Biology, Queen's University. The colony was maintained at 31 ± 1 °C, with a 16:8 h light:dark cycle.

We used a standard preparation for intracellular investigation of locust flight neurons (Robertson and Pearson 1982). After the wings and legs were removed, the locust was pinned dorsal surface uppermost to a cork board. The thorax and anterior portion of the abdomen was opened with a dorsal, midline incision and the thoracic nervous system was exposed by removing the gut and the overlying tissue. Usually nerves 3, 4, 5 (Campbell 1961) were cut to reduce extraneous movement of the preparation. The mesothoracic and metathoracic ganglia were stabilized on a stainless steel platform. The preparation was superfused with locust saline (in mmol/l: NaCl 147, KCl 10, CaCl₂ 4, NaOH 3, Hepes 10, pH 7.2). The temperature of the saline flowing over the ganglia was controlled with a heating coil around a glass pipette that directed the saline into the thoracic cavity. A copper/constantan thermocouple was placed next to the mesothoracic ganglion to monitor the thoracic temperature. Experiments were performed at temperatures in the 20–40 °C range. Flight sequences were initiated with a puff of wind on the head of the animal.

To obtain a monitor of the time of the activity of wing depressor motoneurons, an electromyographic (EMG) electrode (50 µm copper wire, insulated except at the tip) was inserted into one of the thoracic dorsal longitudinal (DL) muscles. The preparation was grounded through an agar/electrolyte bridge. Intracellular recordings were made from neuropil processes of identified motoneurons and interneurons in the mesothoracic and metathoracic ganglia with glass microelectrodes filled with 4% Lucifer Yellow CH in distilled water and the shafts backfilled with 0.5 M lithium chloride (resistance around 100 MΩ). The recordings were usually maintained for more than 10 min, allowing sufficient time to change the temperature. We examined the parameters of the membrane potential oscillations of flight neurons during expression of the flight rhythm by measuring: intraburst spike frequency; bursting frequency; duration of the waveform at 1/2 amplitude; and the amplitude of the waveform. We refer to these as circuit properties as they reflect the activation of the flight circuit. After recording, the neurons were filled with Lucifer Yellow by passing 5 nA of hyperpolarizing current for 5 to 10 min. The thoracic ganglia were

then removed and fixed for 1 h in 4% paraformaldehyde, dehydrated in an ascending alcohol series, cleared in methyl salicylate and viewed in whole mount using a fluorescence microscope. The structures of filled neurons were drawn in dorsal view. The identity of a particular flight motoneuron was confirmed according to morphological and physiological criteria (Hedwig and Pearson 1984).

To measure the cellular properties, recordings were made using 1 M KAc filled glass microelectrodes (resistance around 40 M Ω) inserted in the neuropilar processes of flight motoneurons. A V-I relationship was obtained by measuring the voltage response to different strengths of depolarizing and hyperpolarizing current pulses. The reported input resistance was calculated from the V-I relationship using hyperpolarizing current pulses. In a separate set of experiments, input resistance was measured when normal saline containing 4 mM Ca⁺⁺ but no Mg⁺⁺ was replaced with saline containing no Ca⁺⁺ but 20 mM-Mg⁺⁺. Current thresholds were determined by adjusting the depolarizing stimulus strength until neurons were excited enough to produce a threshold spike. Membrane time constant was estimated by calculating the time required for the membrane potentials to reach 63% of the peak membrane hyperpolarization in response to a hyperpolarizing current pulse.

Paired recordings with two glass-tipped suction electrodes on nerve 1 were used to measure conduction velocities in axons of a flight motoneuron (M81) (Hedwig and Pearson 1984) and the forewing stretch receptor (SR).

All recordings were digitized using a Neurodata neurocorder (DR-886) and stored on VHS videotape for subsequent analysis. Data for each parameter were analysed only if control values at room temperature (around 24 °C) were measured, and if a return to these control values was evident on returning to room temperature. Each successful recording yielded values at room temperature and at least one other temperature.

Least-squares linear regression was used to analyse the parameters of circuit and cellular properties of flight motoneurons. Q_{10} was calculated from the equation of regression line for each parameter. To test difference of data groups in Tables 1 and 3, paired

Student *t*-tests were used. To determine whether temperature had a significant effect on conduction velocity, a one way ANOVA followed by Bonferroni *t*-tests was used. Also a two-way ANOVA was used to analyse the effects of zero Ca⁺⁺/high Mg⁺⁺ and temperature on input resistance of flight motoneurons.

Results

Circuit properties

Activity from 15 flight motoneurons in the mesothoracic or metathoracic ganglia of 14 locusts was recorded during flight. All recordings lasted 10–30 min, allowing sufficient time for changes of temperature. Generally rhythmic activity of locust flight motoneurons was characterized by algebraically summed trains of synaptic potentials with bursts of action potentials riding on the depolarized phases. Changing the thoracic temperature altered the characteristics of the membrane potential waveform and the spiking activity (Fig. 1). Circuit properties were averaged at either 23 °C or 35 °C and their values are compared in Table 1. Since there were variations in the absolute values of these parameters from different

Fig. 1 Intracellular recordings from a depressor (*D*) motoneuron (*upper trace* of each pair) during expression of the flight rhythm at different temperatures (23°, 30°, 35°C). The *lower trace* of each pair is an electromyographic monitor of the activity of dorsal longitudinal (*DL*) motoneurons. Note the increase in burst frequency and intraburst spike frequency with increasing temperature

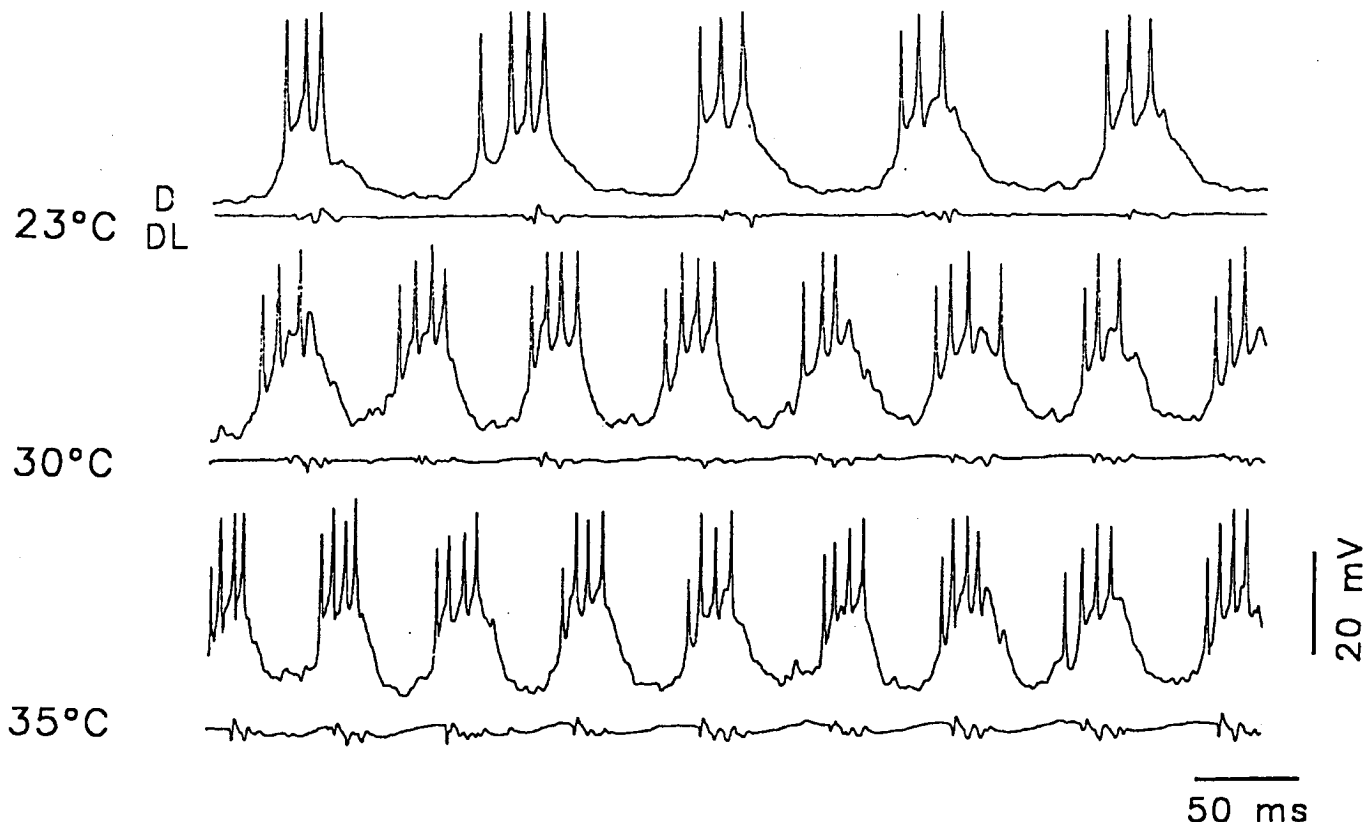
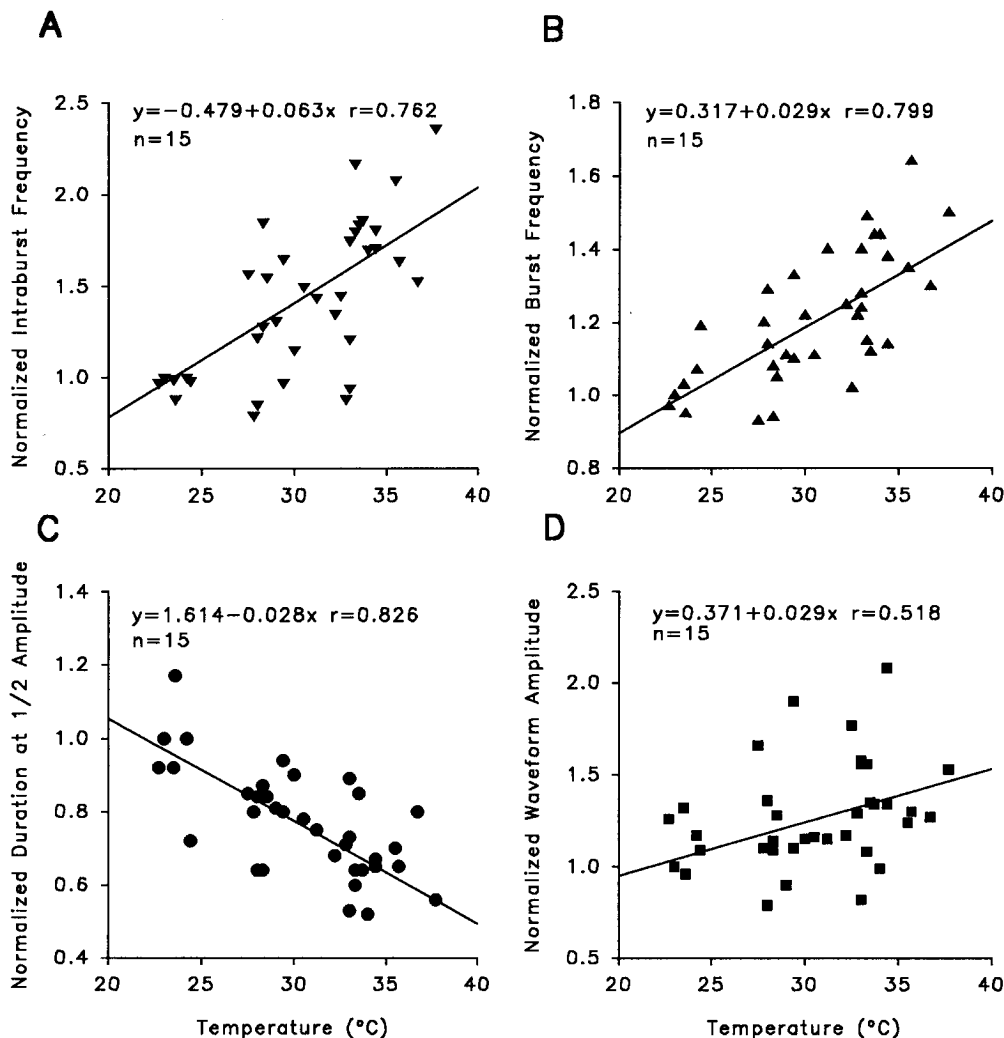


Table 1 Effects of temperature on circuit properties of flight motoneurons ($n=11$)

Temperature (°C)	Burst frequency (Hz)	Intraburst frequency (Hz)	Waveform amplitude (mV)	Duration at 1/2 amplitude (ms)
23° C	9.28±2.86	58.60±24.77	11.12±3.94	57.00±20.55
35° C	11.42±4.47 ^a	95.11±38.77 ^a	16.06±4.57 ^a	40.86±15.88 ^a

Values are presented as mean±standard deviation. Significance was measured with paired *t*-test (^a $P<0.05$)

Fig. 2A–D Normalized parameters of circuit properties of flight motoneurons as a function of temperature. Each data point represents the average value from five consecutive cycles, normalized to the control value at 23 °C. Note that elevated temperature increased the amplitude of the membrane potential waveform, burst frequency and intraburst spike frequency, but decreased the duration at 1/2 amplitude of the waveform. Q_{10} for the intraburst frequency was 1.57, whereas Q_{10} for the burst frequency was 1.28



motoneurons or locusts, each parameter was normalized by dividing by the control value at room temperature. The normalized values were then plotted as a function of temperature (Fig. 2). As temperature increased the intraburst spike frequency, burst frequency and the waveform amplitude increased, whereas the duration at 1/2 amplitude decreased.

Preliminary experiments showed that the circuit properties of flight interneurons ($n=7$) changed with temperature in a similar manner to motoneurons. In interneurons, Q_{10} for the intraburst spike frequency was 1.83, whereas Q_{10} for the burst frequency was 1.34. These Q_{10} values were comparable with the corresponding values of motoneurons (Fig. 2).

Cellular properties

The resting membrane potential (RMP) hyperpolarized when temperature increased (14 motoneurons in 12 locusts) (Fig. 3A). To investigate whether hyperpolarization could have contributed to the observed temperature effects on the circuit properties of flight motoneurons, we hyperpolarized motoneurons (by 5 and 10 mV) in the absence of temperature changes. There was no significant change in the duration and amplitude of membrane potential waveforms during expression of the flight motor pattern (Table 2). The only change was a decrease in the intraburst spike frequency (Table 2).

The V-I relationship of flight motoneurons showed in-

Fig. 3A–D Normalized parameters of cellular properties of flight motoneurons as a function of temperature. Each data point represents the parameter value normalized to the control value at 25 °C. Note that resting membrane potentials of flight motoneurons hyperpolarized on heating and depolarized on cooling. Elevated temperature also decreased the input resistance, membrane time constant, and increased the current threshold of flight motoneurons

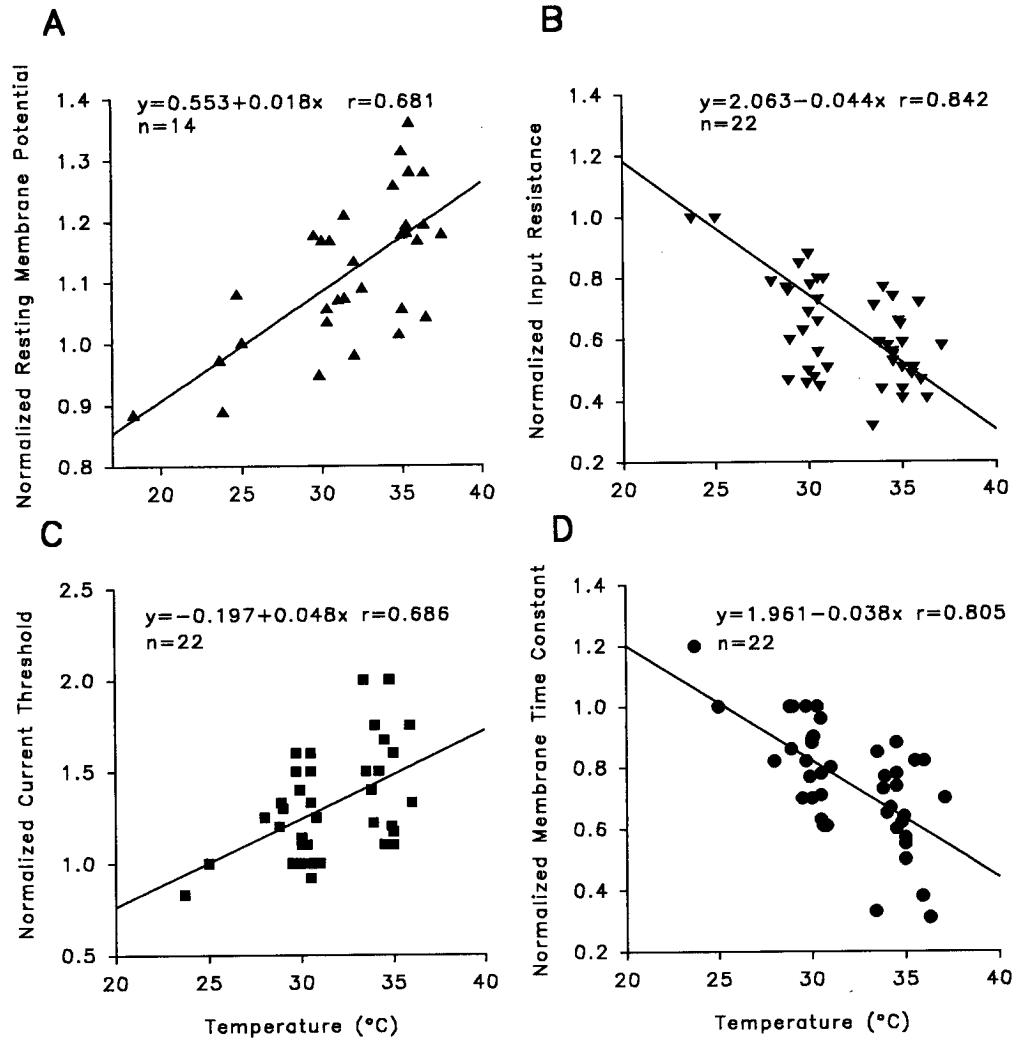


Table 2 Effects of hyperpolarization on circuit properties of flight motoneurons ($n=5$)

	Intraburst frequency (Hz)	Duration at 1/2 amplitude (ms)	Waveform amplitude (mV)
no Hyperpolarization	57.23±14.11 ^a	59.76±17.68	9.81±3.47
5 mV Hyperpolarization	51.84±15.77	61.40±13.79	9.74±3.40
10 mV Hyperpolarization	47.07±12.75 ^a	59.25±10.27	9.22±2.96

Values are presented as mean±standard deviation. Significance was measured with paired *t*-test (^a $P<0.05$)

ward rectification (e.g. Fig. 4B). In all 22 motoneuron recordings from 12 locusts, we found a decrease of input resistance with increasing temperature (Figs. 3B, 4A; Table 3). The current thresholds of flight motoneurons increased with elevated temperature (Fig. 3C). Changes in input resistance might account in large part for the changes in current thresholds described here. Comparing

the membrane potentials at which the first spike was generated at different temperatures, we found that the voltage threshold usually increased only slightly as temperature increased. The membrane time constant was calculated from the response to the largest hyperpolarizing current pulse and was found to decrease with heating (Fig. 3D).

Synaptic input

The reduction of input resistance may have been partially due to an increase in the frequency of synaptic potentials generated from presynaptic neurons that were excited at higher temperatures. To test this, the preparation was superfused with zero Ca^{++} /high Mg^{++} saline to reduce synaptic transmission, and the current stimulation experiments were repeated. Burrows (1975) established that the amplitude of the EPSPs recorded from flight motoneurons declined gradually when normal saline was replaced with one containing no Ca^{++} but 20 mM Mg^{++} . In our experiments, two phenomena were evident: 1) The input resistance of flight motoneurons was always larger

Table 3 Effects of temperature on cellular properties of flight motoneurons

Temperature (°C)	Resting membrane potential (mV) (n=14)	Input resistance (M Ω) (n=22)	Current threshold (nA) (n=22)	Membrane time constant (ms) (n=22)
25° C	-41.07±5.03	3.45±1.23	5.91±2.39	11.45±4.72
35° C	-47.53±6.70 ^a	2.00±0.87 ^a	8.00±2.35 ^a	7.52±3.33 ^a

Values are presented as mean±standard deviation with the number of motoneurons given in parentheses. Significance was measured with paired *t*-test (^a $P < 0.05$)

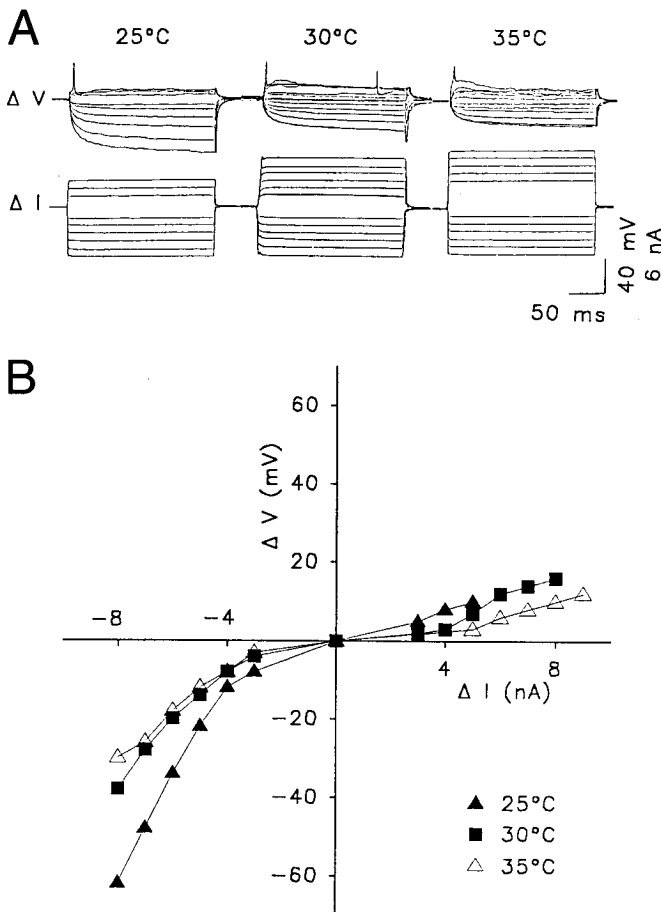


Fig. 4A, B Effect of temperature on the input resistance of locust flight motoneuron. **A** The voltage response of the membrane (upper set of traces) to varying amplitudes of 200 ms duration current pulses (lower set of traces) at different temperatures (25°, 30°, 35° C). The topmost trace in each panel shows a just-threshold action potential generated by the current pulse. Note that elevated temperature slightly increased the voltage threshold. RMP was -42.5 mV. **B** V-I relationship of the same flight motoneuron. Note that the flight motoneuron showed inward rectification, and that the slope of V-I relationship decreased with increasing temperature

in zero Ca⁺⁺/high Mg⁺⁺ saline than in normal saline (Fig. 5). 2) In zero Ca⁺⁺/high Mg⁺⁺ saline, increasing temperature had less effect on the input resistance than in normal saline. The mean slope of the regression lines of normalized input resistance versus temperature in normal saline experiments was significantly higher than the mean slope of the zero Ca⁺⁺/high Mg⁺⁺ saline experiments (Table 4).

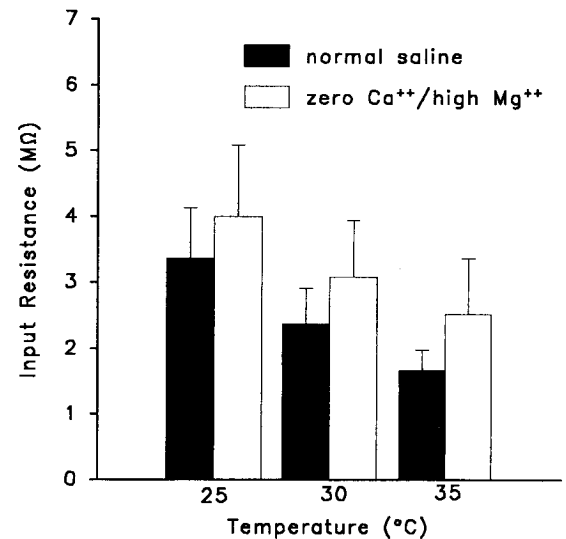


Fig. 5 Comparison of the effect of temperature on input resistance of flight motoneurons in normal saline and in zero Ca⁺⁺/high Mg⁺⁺ saline. Bars represent mean±S.D. (n=7). Note that elevated temperature reduced input resistance in both normal saline and zero Ca⁺⁺/high Mg⁺⁺ saline conditions. Two way ANOVA showed a statistically significant difference in the mean values of input resistance in normal saline and in zero Ca⁺⁺/high Mg⁺⁺ saline after allowing for the temperature effects ($P < 0.01$)

Conduction velocity

At 25 °C, the average conduction velocity of action potentials in flight motoneuron (M81) was 4.40±0.88 m/s (n=7), while that of action potentials in the forewing stretch receptor was 3.33±0.56 m/s (n=7) (Fig. 6). Conduction velocities increased significantly with increasing temperature (Fig. 6). One way ANOVA ($P < 0.001$) followed by Bonferroni *t*-tests revealed significant differences of conduction velocities between 20 °C, 30 °C, and 35 °C ($P < 0.05$).

Q_{10} s for the temperature effects described above are summarized in Table 5.

Discussion

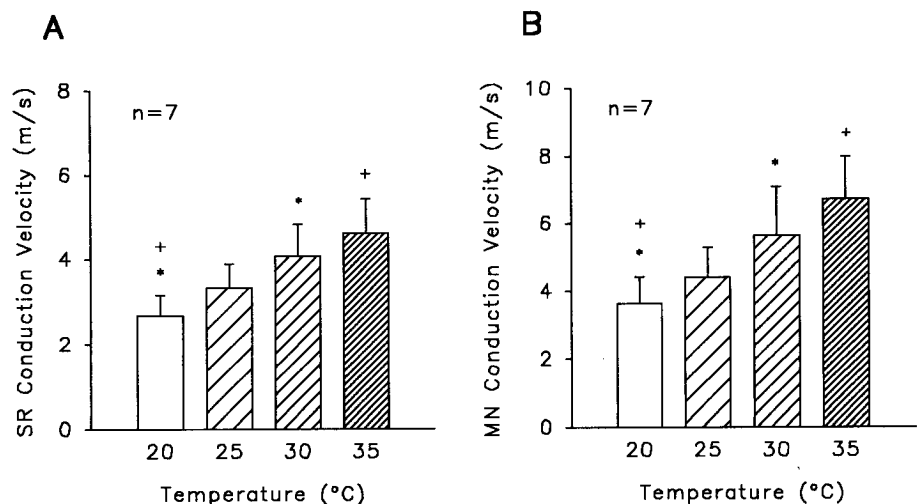
Of the temperature effects on flight motoneurons which we have described in this study, the increase in conduction velocity and the decrease in membrane time con-

Table 4 Slopes and Y-intercepts of linear regressions of normalized input resistances of flight motoneurons as a function of temperature

Neuron number	Slope		Y-intercept	
	Normal saline	Zero Ca ⁺⁺ high Mg ⁺⁺	Normal saline	Zero Ca ⁺⁺ high Mg ⁺⁺
1	-0.075	-0.055	2.833	2.560
2	-0.059	-0.035	2.470	2.053
3	-0.053	-0.047	2.297	2.237
4	-0.070	-0.063	2.717	2.743
5	-0.069	-0.044	2.707	2.447
6	-0.052	-0.052	2.323	2.343
7	-0.050	-0.050	2.203	2.273
mean	-0.061 ^a	-0.049 ^a	2.507	2.379
S.D.	0.010	0.009	0.246	0.227

(^a Indicates significant difference, paired *t*-test, $P < 0.01$)

Fig. 6 Effect of temperature on the conduction velocity of **A** the forewing stretch receptor (SR) and **B** a dorsal longitudinal flight motoneuron (MN). Bars indicate mean \pm S.D. ($n=7$). One way ANOVA revealed a significant temperature effect ($P < 0.001$), and the Bonferroni *t*-tests revealed significant differences ($P < 0.05$) in conduction velocity between 20 °C and 30 °C, and between 20 °C and 35 °C. Asterisks and crosses indicate significant differences

**Table 5** Temperature coefficients (Q_{10} s) of neuronal properties

Neuronal properties	Q_{10}
Resting membrane potential	1.18 ($n=14$)
Input resistance	0.54 ($n=22$)
Current threshold	1.48 ($n=22$)
Membrane time constant	0.62 ($n=22$)
Burst frequency	1.28 ($n=15$)
Intraburst frequency	1.57 ($n=15$)
Waveform amplitude	1.26 ($n=15$)
½ Amplitude duration	0.69 ($n=15$)
SR conduction velocity	1.38 ($n=7$)
MN conduction velocity	1.53 ($n=7$)

stant are likely to be the most important for explaining the increase in the frequency of the centrally generated flight rhythm with increased temperature. An increase of 10 °C increased the conduction velocity of action potentials in a flight motoneuron (M81) from an average of 4.40 m/s at 25 °C to 6.73 m/s at 35 °C. A thermally-induced increase in conduction velocity is not surprising and similar effects have been reported for numerous other preparations (Janssen 1992), including locust neurons (Rowell and Reichert 1986; Burrows 1989). Nevertheless, it is interesting that in a recent study of maturation in the locust flight system, the maturational increase of

wing-beat frequency (from 14 to 22 Hz over about 14 days) correlated with an increase of conduction velocity of flight neurons (Gray and Robertson 1993). In conjunction with our results, this suggests that conduction velocity may be one of the main determinants of wing-beat frequency. Conduction velocity could affect wing-beat frequency not only by reducing time delays between the elements of the circuit (centrally and peripherally), but also by increasing the efficiency of summation of synaptic activity impinging on individual neurons from different presynaptic sources.

We found that increasing temperature could decrease the membrane time constant with a Q_{10} of 0.62. This change would affect the time course of potential changes at the membrane, that, in turn, would affect the spike frequency of flight neurons. The rate and amplitude of action potentials would also be affected (Simmons 1985). The most plausible explanation for the alteration of membrane time constant is increased fluidity of the membrane and conformational changes of leak-channel proteins to decrease membrane resistivity when temperature increases (Janssen 1992). A portion of the effect may be attributable to temperature effects on gated channel properties. For example, examination of the single channel properties of the nicotinic acetylcholine receptor channel from cloned Bc3H-1 cell, confirmed that in-

creases in temperature reduce the mean channel open time (Dilger et al. 1991).

Of the various effects of temperature on neuronal properties which we have described, the factors that would not promote increased wing-beat frequencies at elevated temperatures are the reduction in input resistance and the hyperpolarization of resting membrane potential of flight neurons. Our experimental results on the input resistance ($Q_{10}=0.54$) were consistent with other studies on locust neurons (Heitler et al. 1977; Burrows 1989). Similar changes have been found in *Aplysia* R15 bursting pacemaker neurons (Fletcher and Ram 1991) and crustacean muscle fibres (Stephens and Atwood 1983). Such a reduction in the membrane input resistance of central neurons could account for the increase in the current threshold observed in this study, and the temperature dependent change of PSP amplitude in flight neurons (Robertson 1993). The effect of temperature on input resistance might be due in part to an increase in the frequency of postsynaptic potentials. Flight motoneurons receive synaptic input both from premotor flight interneurons (Robertson and Pearson 1983) and from sensory receptors (Reye and Pearson 1987; Pearson and Wolf 1988). At increased temperatures there are increases in the firing frequencies of both interneurons and stretch receptors (Abrams and Pearson 1982; Pfau et al. 1989). When synaptic transmission was reduced in zero Ca^{++} /high Mg^{++} saline, we found input resistance was larger than that measured in normal saline. Also, with reduced synaptic input, increasing temperature affected input resistance to a lesser extent. We conclude that temperature affected the input resistance of flight motoneurons both by exerting effects on the membrane, and by altering the frequency of synaptic input.

With a 10 °C increase of temperature, we found that the resting membrane potential was hyperpolarized from an average of -41.1 mV at 25 °C to -47.5 mV at 35 °C. This response was qualitatively the same as shown in a previous study (Heitler et al. 1977), but larger in our experiments. It is possible that genetically based variability or a different acclimation regime would produce different results in different studies. Temperature-induced hyperpolarization has been reported in a wide range of animals, including various molluscan neurons (Murray 1966), cat spinal motoneurons (Pierau et al. 1969), and crustacean motor axons (Stephens 1990). Hyperpolarization would lower the excitability of the motoneurons as more current would be required to bring them to their firing thresholds. As expected, we found that hyperpolarizing flight motoneurons during flight sequences significantly decreased their intraburst spike frequency. However, motoneurons are considered not to be involved in the timing of the flight rhythm so it was not surprising that hyperpolarization had no effect on the burst frequency. Also, hyperpolarization in itself had no significant effect on the amplitude, nor on the duration at 1/2 amplitude, of the waveforms. Temperature effects on these parameters must be effected via an alternative route.

Temperature may also affect other neuronal properties

with important roles in central pattern generation. These properties include endogenous bursting and plateau potential generation of flight interneurons. It has been reported recently that octopamine can induce flight neurons to produce plateau potentials in response to synaptic inputs from the tegulae or as a result of short current pulse stimuli (Ramirez and Pearson 1991a, b, c). It was further proposed that these plateau potentials and the endogenous bursting properties of flight interneurons might contribute to the production of the wind-induced rhythmic flight activity in deafferented and intact animals. Interestingly, we observed an increase in the amplitude of the membrane potential waveforms underlying bursts in response to an increase in temperature. This cannot be attributed to changes in individual PSPs, for they reduce in amplitude with the same temperature changes (Robertson 1993). Nor can it be attributed to the temperature-induced hyperpolarization, because equivalent hyperpolarization does not significantly affect the amplitude of membrane potential waveforms. In addition, the observed decrease in input resistance would reduce these amplitudes in the absence of other changes. One possibility is that increases in the firing rate of presynaptic interneurons play an important role. However, we have not yet examined the effect of temperature on endogenous mechanisms for burst generation. It is conceivable that these intrinsic cellular properties are more resistant to temperature changes and thus can compensate for temperature-induced variations in synaptic potentials and neuronal excitability.

The locust flight circuit operates by virtue of a series of excitation and propagation processes and, in the intact flight system, these include the peripheral components of proprioceptors and muscles. It has been shown that the discharge frequency of the forewing stretch receptor at a given stretch, and the saturation frequency, both depend critically on temperature (Pfau et al. 1989). In this study, we found that the Q_{10} for SR conduction velocity was 1.38. We also found that the Q_{10} for burst frequency changes of the deafferented preparation was 1.28. However, the slope of a linear relationship between temperature and rhythm frequency has been reported as 0.27 for intact animal and 0.29 for deafferented preparation (Foster and Robertson 1992). This results in a Q_{10} for intact wing-beat frequency of 1.15 and a Q_{10} for the deafferented flight rhythm of 1.19 respectively (differing because the repetition frequencies are lower in a deafferented preparation). Examination of Table 3 in Foster and Robertson (1992) suggests that pooling of the results from 6 deafferented preparations has artefactually reduced the slope of the relationship of rhythm frequency to temperature. Averaging the slopes given in Table 3 of that paper results in a slope of 0.35 and a Q_{10} of 1.23 which is much closer to the present result. Thus it appears that wing-beat frequency in the intact locust may be temperature-compensated to a greater extent than rhythm frequency in the deafferented preparation. This might be because the flight system is already cycling near a theoretical upper limit, but it is also possible that the periph-

eral feedback loops (Pearson and Ramirez 1990) are inherently more resistant to temperature fluctuations and can stabilize the wingbeat frequency. Considering the fact that temperature also affects insect muscle activity (Neville and Weis-Fogh 1963; Koch et al. 1988), it is not precluded that flight muscle activity might also be involved in automatic compensation.

Temperature-induced changes of neuronal properties differed qualitatively with each other, indicating that automatic compensation exists within central flight circuitry to stabilize output frequency. Based on our present results, we propose that elevating temperature increases the frequency of the centrally generated rhythm by changing those cellular properties related to temporal summation of flight neuron activity in the circuit. These are the increase in conduction velocity ($Q_{10}=1.53$), and the decrease of membrane time constant ($Q_{10}=0.62$). We further propose that the reduction in input resistance ($Q_{10}=0.54$) and hyperpolarization of resting membrane potential ($Q_{10}=1.18$), would decrease the excitability of flight neurons, and are involved in automatic compensation.

The output frequency of a central pattern generator is determined by a complex spatial and temporal interaction of multiple processes at the cellular, synaptic and network levels. Modulation of any of these building blocks can alter network operation (Getting 1989). Relative involvement of each factor in setting the pattern frequency is likely to be different in different invertebrate species (Selverston and Moulins 1987). This difference could result in different thermosensitivities of neuronal circuitry. For the locust flight system, the mechanisms determining the output frequency remain to be elucidated. However, a comparative analysis of temperature and neuromodulator effects on flight motor pattern generation would help in understanding which processes predominate in setting rhythm frequency.

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